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Geographic variation in leaf essential oils and RAPDs of *Juniperus polycarpos* K. Koch in central Asia

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Abstract

Variations in the composition of the leaf essential oils and DNA fingerprints (RAPDs) of Juniperus excelsa, J. polycarpos, J. seravschanica, and J. turcomanica were examined. Juniperus procera was also included in the analyses to aid in determining the specific status of J. polycarpos. Based on these analyses, J. polycarpos is recognized as a distinct species from J. excelsa. The common, multi-seeded juniper of central Asia is J. polycarpos. Juniperus seravschanica and J. turcomanica are treated as part of the J. polycarpos complex but are not recognized as formal taxonomic groups at this time. The Balochistan, Pakistan juniper, usually called J. excelsa var. polycarpos or J. macropoda should be referred to as J. polycarpos in the future. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Juniperus; Cupressaceae; Essential oils; Terpenes; RAPD; DNA fingerprinting; Systematics

1. Introduction

Previously, I reported on the leaf oils and DNA fingerprinting for the multi-seeded junipers of the eastern hemisphere (Adams, 1999). However, recent field work by the author in Armenia and Turkmenistan seemed to indicate that previous collections from the Tbilisi Botanic Garden were mis-identified. The Tbilisi plants, identified as *J. excelsa* var. *polycarpos* (K. Koch) Silba, appeared to be, morphologically, more similar to *J. excelsa* M.-Bieb. from Greece than to the new collections of *J. polycarpos* K. Koch in Armenia. In addition, the new collections of *J. turcomanica*

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B. A. Fedtsch. from Turkmenistan appeared to be very similar to *J. polycarpos* from Armenia. So it seemed appropriate to re-analyze the leaf essential oils and DNA fingerprints for these new collections and compared them with the previous work (Adams, 1999). The literature on the taxonomy and leaf essential oils has been recently reviewed (Adams, 1999).

2. Materials and methods

Specimens used in this study: J. excelsa, Adams 5983-5887, 8785-8786-7 km w of Lemos, Greece; J. excelsa var. "polycarpos", Adams 6139-6141 — Tbilisi Botanic Garden, Georgia, CIS; J. seravschanica, Adams 8224-8226 — 2 km s Dzhabagly, Kazakstan (not Kyrgystan as previously reported, Adams, 1999); J. excelsa var. polycarpos, Adams 8483-8486 — Quetta, Balochistan, Pakistan; J. turcomanica, Adams 6713-6716 — Almaty Botanic Garden, Kazakstan (origin = near Ashgabad, Turkmenistan) and Adams 8757-8760, Kopet Mts., Turkmenistan. In order to evaluate specific level differences, J. procera from east Africa was included in these analyses: J. procera, Adams 6184, 6185 — 40 km w of Addis Ababa, Ethiopia, and Adams 5333-5335 — 38 km nw of Nairobi, Kenya. Voucher specimens for all collections are deposited at SRCG.

Fresh leaves (200 g fresh wt.) were steam distilled for 2h using a circulatory Cleavenger apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at — 20°C until analyzed. The extracted leaves were oven dried (48 h, 100°C) for determination of oil yields. After initial GCMS analyses, composite oil samples were made for each of the taxa in this study. These composite (average) oil samples were then subjected to GCMS for compound identification and quantitation by TIC.

The essential oils were analyzed on a Finnigan ion trap (ITD) mass spectrometer, model 800, directly coupled to a Varian 6500 gas chromatograph, using a J & W DB-5, 0.26 mm × 30 m, 0.25 µm coating thickness, fused silica capillary column (see Adams, 1995for operating details). Identifications were made by library searches of our volatile oil library, LIBR(TP) (Adams, 1995), using the Finnigan library search routines based on fit and purity, coupled with retention time data of reference compounds.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by the hot CTAB protocol (Doyle and Doyle, 1987) with 1% (w/v) PVP added to the extraction buffer. The RAPDs analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Colombia (5′–3′): 116: TAC GAT GAC G; 134: AAC ACA CGA G; 153: GAG TCA CGA G; 204: TTC GGG CGC T; 212: GCT GCG TGA C; 218: CTC AGC CCA G; 239: CTG AAG CGG A; 244: CAG CCA ACC G; 250: CGA CAG TCC C; 265: CAG CTG TTC A; 327: ATA CGG CGT C; 338: CTG TGG CGG T; 346: TAG GCG AAC G; 347: TTG CTT GGC G; 375: CCG GAC ACG A.

PCR was performed in a volume of 12.5 μl containing 50 mM Tris-HCl (pH 9), 2.0 mM MgCl₂, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each DNTPs, 0.36 μM primers, 0.25 ng genomic DNA, and 0.5 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 38°C (2 min), 72°C (2 min), 91°C (1 min). Two additional steps were used: 38°C (2 min) and 72°C (5 min) for final extension. Bands were scored in 4 classes: very bright (=6); medium bright (=5), faint (=4) and absent (=0). See Adams and Demeke (1993) for details on electrophoresis and RAPD band scoring.

Similarity measures were computed as presence/absence matches as well as using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (=Gower metric, Gower, 1971; Adams, 1975a,b). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966).

3. Results and discussion

The compositions of the volatile leaf oils are given in Table 1. Notice the large amounts α -pinene in the plants from Armenia and Turkmenistan. Myrcene is a large component in the oils from Kazakstan and Pakistan. Several compounds distinguish the *J. excelsa* samples: decadienal isomer (KI 1312), *trans*-cadina-1(6),4-diene, cubebol, 1-epi-cubenol, and KI 1666 (Table 1). Compounds that distinguish *J. polycarpos* (including *J. turcomanica* and *J. seravschanica* for this discussion) are: hexyl 3-methyl butanoate, δ -elemene, γ -cadinene, elemol, germacrene B, germacrene D-4-ol, α & β -eudesmols, and KI 1688 (Table 1). Several diterpenes are unique to *J. procera* (Table 1) and show its separation from *J. excelsa* and the other junipers.

In order to assimilate the overall trend in the volatile leaf oils, similarities were using presence/absence of terpenes. Presence/absence appears to be better suited for the analysis of differences among species, whereas quantitative matching is more sensitive for use at the infraspecific level (Adams, 1999). Fig. 1 shows the clustering based on presence/absence matching for *J. excelsa*, *J. procera* and putative *J. polycarpos* populations using 106 terpenes. A major difference in this analysis and the previous analysis (Adams, 1999) is that the putative *J. excelsa* var. "polycarpos" from the Tbilisi Botanic Garden (EG) clusters closely with *J. excelsa* from Greece and not with the field collected *J. polycarpos* from Armenia. The collections from Kazakstan (*J. seravschanica*), Pakistan (*J. excelsa* var. polycarpos), and Turkmenistan (*J. turcomanica*) all cluster with *J. polycarpos* from Armenia (Fig. 1) and not with *J. excelsa*. This supports the concept that *J. polycarpos* is a variable species ranging from Armenia to Pakistan. In addition, *J. procera* is very distinct as is *J. excelsa*.

To examine geographic variation within the *J. polycarpos-seravschanica-turcomanica* complex, *J. excelsa* and *J. procera* were removed from the data set and quantitative matches were used to compute similarities among the junipers from four

Table 1 Comparisons of the per cent total oil for leaf essential oils for *J. excelsa* — Greece (EG), *J. excelsa*, Tbilisi Botanic Garden (ET), various populations of J. *polycarpos*: Armenia, L. Sevan (AS); Turkmenistan, Kopet Mts. (TK), Alma Ata Botanic Garden (ex. Ashgabad, Turkmenistan, TA), Kazakstan, Talasskiy Mtns. (KT), Pakistan, Quetta (PQ), and *J. procera*, east Africa (PR). Components that tend to separate the species are highlighted in boldface^a

| KI | Compound | EG | ET | AS | TK | TA | KT | PQ | PR |
|--------------|------------------------------------|------|------|------|------|------|------|------|------|
| 926 | tricyclene | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.3 | 0.1 | t |
| 931 | α-thujene | | | t | t | t | 0.6 | 0.4 | t |
| 939 | α-pinene | 22.5 | 26.5 | 68.4 | 68.8 | 59.7 | 44.4 | 15.5 | 12.5 |
| 953 | α-fenchene | 0.2 | T | t | t | t | _ | 0.2 | 0.1 |
| 953 | camphene | 0.5 | 1.0 | 0.2 | 0.1 | 0.5 | 0.5 | 0.2 | 0.1 |
| 957 | thuja-2,4(10)-diene | 0.1 | _ | t | t | t | _ | | - |
| 975 | verbenene | t | | | _ | | _ | _ | _ |
| 976 | sabinene | t | 0.1 | 0.2 | 0.1 | 0.4 | 0.9 | 0.5 | t |
| 978 | 1-octen-3-ol | | | | _ | | _ | _ | 0.3 |
| 980 | β -pinene | 0.6 | 1.0 | 0.5 | 0.6 | 1.8 | 2.2 | 1.2 | 1.2 |
| 991 | myrcene | 1.9 | 2.2 | 1.2 | 1.5 | 3.7 | 19.2 | 20.7 | 1.2 |
| 1005 | α-phellandrene | 0.1 | 0.1 | _ | | t | 0.1 | 0.1 | |
| 1011 | δ -3-carene | 2.3 | 0.4 | t | t | t | | 3.5 | 6.1 |
| 1018 | α-terpinene | 0.1 | 0.1 | t | t | t | 0.1 | 0.1 | t |
| 1026 | p-cymene | 0.4 | 0.2 | 0.1 | 0.1 | 0.2 | 0.1 | 0.7 | t |
| 1028 | sylvestrene | | _ | | | | _ | _ | 0.1 |
| 1031 | limonene | 22.6 | 5.5 | 1.2 | 1.5 | 1.8 | 4.4 | 9.0 | 0.2 |
| 1031 | β-phellandrene | t | | | | 0.1 | 0.5 | 1.0 | 0.8 |
| 1032 | 1,8-cineole | | t | | | _ | | | t |
| 1050 | (E)-β-ocimene | t | | t | t | t | t | 0.2 | t |
| 1062 | γ-terpinene | 0.6 | 0.9 | 0.2 | 0.3 | 0.6 | 1.4 | 1.3 | t |
| 1068 | cis-sabinene hydrate | _ | _ | _ | _ | _ | 0.1 | 0.2 | _ |
| 1068 | fenchone | | | t | t | t | t | t | _ |
| 1088 | terpinolene | 0.9 | 1.1 | 0.4 | 0.5 | 1.3 | 1.5 | 1.7 | 1.1 |
| 1097 | trans-sabinene hydrate | | | ٠ | | t | | _ | _ |
| 1098 | linalool | _ | 0.1 | 0.1 | t | t | 0.5 | 0.7 | 0.5 |
| 1103 | isopentyl-isovalerate | | | t | t | t | | _ | _ |
| 1110 | 1,3,8-p-menthatriene | | | _ | | | _ | _ | t |
| 1112 | endo-fenchol | 0.2 | | | | t | | | _ |
| 1114 | trans-thujone(= β -thujone) | | | t | t | 0.2 | 0.1 | | |
| 1121 | cis-p-menth-2-en-1-ol | 0.1 | | - | _ | | t | _ | t |
| 1125 | chrysanthenone | | | t | t | t | | _ | _ |
| 1125 | α-campholenal | 0.1 | 0.2 | 0.2 | 0.1 | 0.2 | t | 0.1 | t |
| 1134 | cis-limonene oxide | | | | | | _ | | 0.1 |
| 1139 | trans-pinocarveol | 0.2 | 0.3 | 0.1 | 0.1 | t | _ | t | t t |
| 1143 | camphor | 0.2 | 0.3 | t.1 | 0.1 | 1.7 | t | t | t |
| 1143 | cis-sabinol ^b | 0.3 | 0.2 | 0.4 | 0.3 | 1./ | ι | 0.2 | 0.2 |
| 1143 | trans-verbenol | | 0.5 | 0.4 | 0.3 | t | t | 0.2 | t.2 |
| 1143 | | | 0.3 | _ | _ | ι | ι | _ | 0.1 |
| 1160 | p-mentha-1,5-dien-8-ol | | | | | _ | | | U. I |
| | trans-pinocamphone | _ | | t | _ | _ | | | _ |
| 1163 1165 | pinocarvone | _ | 0.1 | t | | | _ | | 0.2 |
| | borneol | _ | t | | t | t | t | t | |
| 1167 | δ -terpineol | | | t | t | | t | t | t |
| 1173 | cis-pinocamphone | -0.2 | | t | | | | | |
| 1177 | terpinen-4-ol | 0.2 | 0.2 | 0.1 | t | 0.2 | 0.4 | 0.3 | 0.1 |

Table 1— continued

| KI | Compound | EG | ET | AS | TK | TA | KT | PQ | PR |
|------|--------------------------------------|-----|-----|-------------|-------|-----|-----|-----|-----|
| 1178 | naphthalene | t | t | 0.1 | 0.4 | t | t | t | |
| 1180 | m-cymen-8-ol | _ | | _ | _ | _ | _ | _ | 0.1 |
| 1183 | p-cymen-8-ol | | _ | t | _ | _ | | _ | t |
| 1185 | trans-p-mentha-1(7),8-dien-2-ol | 0.1 | _ | | _ | _ | _ | _ | _ |
| 1189 | α-terpineol | t | 0.1 | t | t | 0.2 | 0.1 | t | 0.5 |
| 1191 | hexyl butyrate | _ | | _ | _ | 0.1 | _ | _ | |
| 1204 | verbenone | 0.1 | 0.1 | 0.1 | 0.1 | t | | | _ |
| 1217 | trans-carveol | 0.1 | 0.1 | t | t | _ | _ | _ | |
| 1220 | endo-fenchyl acetate | 0.3 | 0.1 | _ | _ | | _ | _ | _ |
| 1242 | hexyl 3-methyl butanoate | _ | | 0.1 | 0.2 | 0.4 | _ | t | - |
| 1257 | 4Z-decen-1-ol | _ | _ | _ | _ | 0.2 | _ | _ | _ |
| 1274 | unknown, <u>79</u> ,91,105,147,FW162 | _ | | _ | _ | _ | - | _ | 0.3 |
| 1285 | bornyl acetate | 0.4 | 0.9 | 0.2 | 0.2 | 0.7 | 1.0 | 0.6 | 0.4 |
| 1286 | linalool oxide acetate(pyranoid) | 0.2 | 0.1 | | _ | _ | _ | _ | - |
| 1290 | trans-sabinyl acetate | _ | _ | _ | _ | _ | _ | 0.1 | _ |
| 1312 | decadienal isomer? | 3.3 | 5.6 | _ | _ | _ | _ | | |
| 1319 | 2E,4E-decadienal | _ | | _ | | t | t | t | _ |
| 1339 | δ -elemene | _ | _ | t | 0.1 | t | t | t | _ |
| 1376 | α-copaene | | 0.2 | _ | t | _ | | | _ |
| 1383 | β -bourbonene | 0.1 | | _ | _ | _ | _ | _ | |
| 1381 | hexyl n-hexanoate | _ | | _ | 0.1 | 0.7 | | | _ |
| 1389 | β -cubebene | 0.1 | 0.1 | _ | 0.1 | _ | _ | _ | _ |
| 1409 | α-cedrene | _ | t | _ | _ | _ | | | _ |
| 1409 | 1,7-di-epi-β-cedrene | 1.6 | 0.7 | 1.3 | _ | _ | 0.2 | 1.4 | _ |
| 1418 | (E)-caryophyllene | | 0.1 | 0.3 | 0.4 | _ | 0.1 | 0.2 | 0.5 |
| 1418 | β -cedrene | 0.9 | 0.5 | ***** | _ | _ | 0.1 | 0.2 | |
| 1429 | cis-thujopsene | 0.3 | 0.2 | 0.2 | | _ | 0.2 | 0.4 | _ |
| 1446 | cis-muurola-3,5-diene | 0.2 | 0.6 | _ | 0.2 | | t | | |
| 1454 | α-humulene | 0.2 | 0.2 | | 0.1 | _ | | _ | 0.7 |
| 1458 | E-β-farnesene | 0.2 | 0.1 | 0.1 | _ | _ | _ | 0.1 | |
| 1461 | cis-muurola-4(14),5-diene | _ | _ | 0.1 | 0.2 | _ | _ | 0.1 | _ |
| 1466 | β -acoradiene | 0.1 | t | 0.1 | _ | _ | | t | _ |
| 1473 | trans-cadina-1(6),4-diene | 0.4 | 0.8 | | _ | _ | _ | _ | _ |
| 1477 | γ-muurolene | _ | t | _ | 0.2 | 0.1 | t | _ | _ |
| 1480 | germacrene D | 0.9 | 1.7 | 0.2 | 0.8 | 0.8 | 0.1 | 0.2 | 0.3 |
| 1491 | trans-murrola-4(14),5-diene | 0.4 | 1.4 | | 0.1 | 0.1 | t | _ | |
| 1493 | epi-cubebol | | 1.3 | | t | 0.2 | t | t | |
| 1499 | γ-amorphene | | | | | _ | _ | t | _ |
| 1499 | α-muurolene | 0.2 | 0.1 | and Service | 0.3 | 0.2 | 0.2 | 0.2 | |
| 1499 | bicyclogermacrene | | | t | | | _ | t | _ |
| 1502 | cuparene | | | | | | | t | _ |
| 1503 | germacrene A | | | | 0.1 | 0.1 | _ | _ | _ |
| 1509 | β -bisabolene | | _ | t | | _ | _ | t | _ |
| 1512 | α-alaskene | 0.3 | t | 0.1 | _ | _ | _ | 0.4 | |
| 1513 | γ-cadinene | _ | _ | 0.2 | 1.1 | 0.9 | 0.4 | 0.4 | |
| 1513 | cubebol | 0.8 | 2.6 | _ | _ | _ | | - | _ |
| 1521 | cis-calamenene | t | _ | _ | ***** | | _ | _ | _ |
| 1524 | δ -cadinene | 0.7 | 1.5 | 0.3 | 1.4 | 1.2 | 1.0 | 0.7 | _ |
| | | 0.2 | | | | | | | |

Table 1—continued

Table 1- continued

| KI | Compound | EG | ET | AS | TK | TA | KT | PQ | PR |
|------|--|------|------|--------|-----|-----|------|------|--|
| 1532 | trans-cadina-1(2),4-diene | t | 0.2 | _ | t | _ | t | _ | _ |
| 1538 | α-cadinene | | _ | _ | 0.2 | 0.2 | 0.1 | 0.1 | |
| 1549 | elemol | _ | _ | t | 0.2 | 1.9 | 0.5 | 0.5 | 4.3 |
| 1556 | germacrene B | _ | _ | 0.7 | 1.6 | 1.8 | 0.4 | 0.6 | |
| 1574 | germacrene D-4-ol | _ | t | 0.4 | 3.0 | 1.5 | 0.5 | 2.0 | 0.1 |
| 1581 | caryophyllene oxide | _ | _ | _ | _ | _ | _ | _ | 0.5 |
| 1587 | sesquiterpene, FW220? | 1.9 | 1.7 | 0.8 | _ | | 1.0 | 1.7 | _ |
| 1596 | cedrol | 28.1 | 30.8 | 19.0 | t | _ | 14.6 | 26.4 | _ |
| 1606 | humulene epoxide II | t | _ | _ | _ | _ | _ | _ | 0.5 |
| 1606 | β -oplopenone | t | _ | t | 0.2 | 0.2 | _ | _ | _ |
| 1607 | 4E-tridec-6-yne ^b | | 0.6 | | | | | | |
| 1627 | 1-epi-cubenol | 1.6 | 2.2 | t | _ | _ | _ | _ | and the same of th |
| 1630 | γ-eudesmol | | _ | _ | t | 0.5 | | | 1.4 |
| 1640 | epi-α-cadinol | t | 0.3 | 0.1 | 0.7 | 0.9 | 0.5 | 0.4 | |
| 1640 | epi-α-muurolol | t | 0.5 | t | 0.7 | 0.9 | 0.1 | 0.1 | _ |
| 1645 | α-muurolol | t | 0.2 | _ | 0.2 | 0.4 | t | t | |
| 1649 | β -eudesmol | | | t | t | 0.8 | t | 0.1 | 2.3 |
| 1652 | α-eudesmol | _ | _ | t | t | 0.5 | 0.1 | t | 3.8 |
| 1653 | α-cadinol | t | 0.2 | 0.2 | 1.6 | 3.2 | 0.9 | 0.8 | |
| 1666 | bunesol | _ | _ | t | t | 0.4 | _ | 0.1 | 1.3 |
| 1666 | unknown(57,41,85,79,136) | 0.6 | 2.4 | _ | _ | _ | _ | _ | _ |
| 1685 | eudesma- $\overline{4(15)}$,7-dien-1- β -ol | | | _ | | _ | | | 0.2 |
| 1688 | sesquiterpene alcohol, FW 222 | | | 0.3 | 0.9 | 3.6 | 0.4 | _ | _ |
| 1688 | cadinol isomer | | | _ | t | 1.2 | | | |
| 1789 | 8-α-acetoxyelemol | | _ | | _ | | _ | _ | 3.5 |
| 1809 | unknown(43,79,71,99,136,252) | | 0.6 | _ | _ | t | _ | | |
| 1930 | rosa-5,15-diene | _ | - | | | | _ | _ | 0.4 |
| 1961 | sandaracopimara-8(14),15-diene t | _ | _ | | _ | _ | _ | _ | 0.3 |
| 1989 | manoyl oxide | T | 0.2 | 0.1 | 0.1 | _ | | 0.3 | 0.5 |
| 2054 | abietatriene | T | 0.4 | t | t | 0.3 | _ | t | 1.3 |
| 2080 | abietadiene | 0.3 | 2.2 | 0.3 | 0.7 | _ | _ | 0.2 | 15.4 |
| 2103 | diterpene, 41,79,191,257,FW286? - | _ | _ | | _ | _ | _ | _ | 2.6 |
| 2147 | abieta-8(14),13(15)-diene ^b | _ | _ | _ | _ | _ | | | 0.3 |
| 2181 | diterpene, 41, 91, 271, 257, FW286 - | _ | _ | ****** | _ | | _ | _ | 0.8 |
| 2278 | sempervirol | | | | | _ | _ | _ | 0.6 |
| 2288 | 4-epi-abietal | 0.1 | 1.2 | 0.6 | 1.1 | 1.7 | _ | 1.0 | 1.8 |
| 2293 | diterpene,41,55,255,269,FW284? | _ | | | | _ | _ | _ | 1.0 |
| 2302 | abieta-7,13-dien-3-one | | 0.1 | | | 0.2 | | _ | _ |
| 2302 | trans-totarol | _ | _ | | _ | | _ | _ | 21.4 |
| 2325 | trans-ferruginol | | 0.3 | _ | | | | | 3.4 |

^aKI=Kovat's Index on DB-5(=SE54) column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

natural populations (AS, TK, KT, PQ). Clustering using these 94 terpenes resulted in two groups: Armenia–Turkmenistan (AS–TK, 0.804) and Kazakstan–Pakistan (KT–PQ, 0.811) (Fig. 2). The two groups were joined by linkage of Armenia (AS) and Pakistan (PQ) at a similarity of 0.770.

^bTentatively identified.

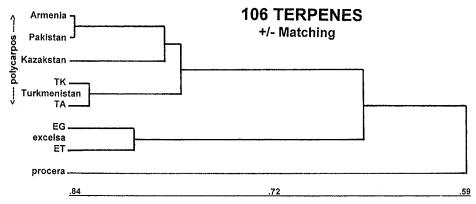


Fig. 1. Minimum spanning network based on 106 terpenoids, with similarities computed as presence/absence matches. Notice the differences between the *J. polycarpos* group and *J. excelsa*. See text for discussion.

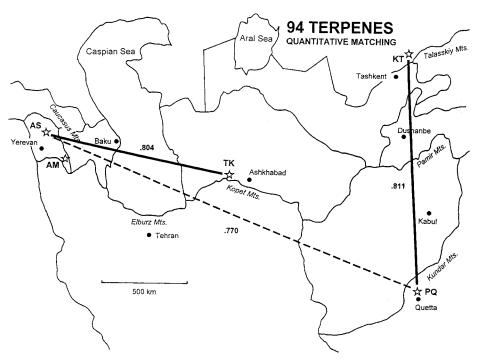


Fig. 2. Minimum spanning network for 4 populations of the *J. polycarpos-turcomanica-seravschanica* complex. Two barely defined groups are present: Armenia (AS) — Turkmenistan (TK) and Pakistan (PQ) — Kazakhstan (KT) and they unite at 0.770.

A preliminary inspection of the RAPD bands revealed that several bands were merely local polymorphisms (i.e., present in only one individual), several bands were present in several individuals but not in two individuals of the same taxon, some bands showed fidelity (present in both samples of a taxon) in some taxa but not in

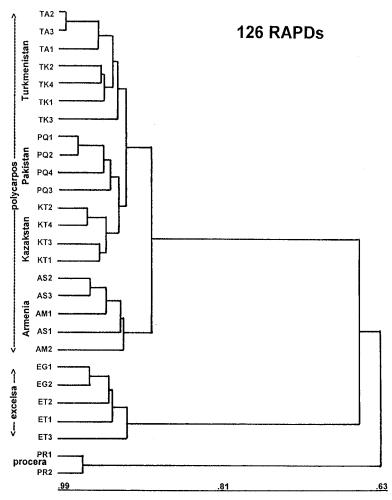


Fig. 3. Minimum spanning network based on 126 RAPD bands. The cultivated *J. excelsa* from Tbilisi (ET1,2,3) cluster closely with the J. excelsa from Greece (EG1,2). Three groups are apparent: *J. excelsa*, *J. polycarpos* and *J. procera*.

others and some bands showed both fidelity and discrimination characteristics. DNA fingerprinting can be used at several taxonomic levels, depending on the primer used; ranging from intra-generic levels (Adams and Demeke, 1993) to distinguishing between individuals (Demeke et al., 1992). It is important to realize that the presence of a band could be very significant when searching for a marker for disease resistance (for example), but in the present instance, fidelity is needed to screen out this "individual variation". Thus, only the bands showing fidelity within populations were used.

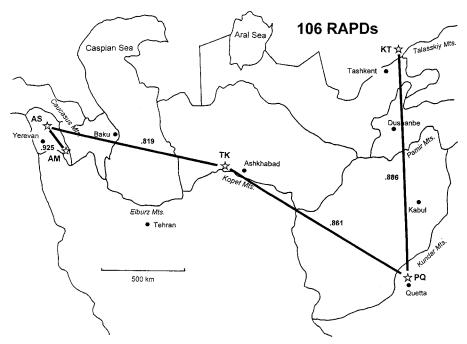


Fig. 4. Minimum spanning network based on 106 RAPDs for the 5 natural populations sampled. Note the high similarity between AS and AM in Armenia, and between TK-PQ-KT.

Clustering based on 126 RAPDs revealed three groups: *J. excelsa*, *J. procera* and the *J. polycarpos-seravschanica-turcomanica* complex (Fig. 3). Note that these three groups are clustered at about the same level (Fig. 3). This supports the concept of three species: *J. excelsa*, *J. polycarpos* and *J. procera*. This is in deference to the previous work (Adams, 1999) in which the plants from Tbilisi Botanic Garden were used as reference materials for *J. excelsa* var. *polycarpos*. The present study clearly shows that the material from the Tbilisi Botanic Garden is *J. excelsa*.

Analysis of geographic variation within the *J. polycarpos* complex was accomplished by removing *J. excelsa* and *J. procera* individuals from the data set. This resulted in 106 RAPDs. A minimum spanning network is shown in Fig. 4. The Armenia populations are somewhat differentiated from the Turkmenistan population, whereas the Pakistan and Kazakstan populations are very similar, followed by the Turkmenistan population (Fig. 4.). Note that all of these populations are found in mountain ranges that likely act as islands in restricting gene flow.

Principal coordinate analysis of the 106 RAPDs and all individuals obtained from natural populations revealed an east—west trend (Fig. 5). In addition, one can see the differentiation of the Pakistan, Turkmenistan and Kazakstan populations. The juniper from the Kopet Mts., Turkmenistan has been recognized at *J. turcomanica* and the juniper from the Talasskiy Mts., Kazakstan has been recognized as *J. seravschanica* (Adams, 1999). Both Farjon (1992) and Silba (1986, 1990) considered

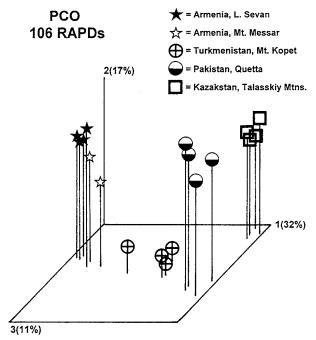


Fig. 5. Principal coordinate analysis based on 106 RAPDs. The individuals cluster into 4 populations, showing an east-west gradient. See text for discussion.

J. seravschanica and J. turcomanica to be synonyms of J. excelsa var. polycarpos. The Balochistan, Pakistan juniper is still called J. excelsa (Ciesla et al., 1998). With the present data sets, it appears prudent to recognize J. turcomanica and J. seravschanica as part of J. polycarpos. Thus, the Balochistan, Ziarat juniper should be referred to as J. polycarpos not J. excelsa or J. macropoda. The classification of J. turcomanica and J. seravschanica as varieties of J. polycarpos is suggested but not well supported by the data in this study. The synonymy below follows Farjon (1998):

J. polycarpos K. Koch, Linnnea 22: 303 (1849)

Sabina polycarpos (K. Koch) Antoine, Cupress.-Gatt.: 47 (1857).

- J. excelsa M.-Bieb. var. polycarpos (K. Koch) Silba, Phytologia Mem. 7:34 (1984).
- J. macropoda Boiss., Fl. Orient. 5: 709 (1884).
- J. seravschanica Kom., Bot Zurn. (Moscow & Leningrad) 17: 481 (1932).
- J. polycarpos K. Koch var. seravschanica (Kom.) Kitam., Add. & Corr. Fl. Afghan.: 68 (1966).

Sabina seravschanica (Kom.) Nevski, Trudy Bot. Inst. Akad. Nauk. S.S.S.R., ser. 1, Fl. Sist. Vyss. Rast. 4: 245 (1937).

- J. excelsa M.-Bieb. subsp. seravschanica (Kom.) Imkhan., Bot Zurn. 75(3): 407 (1990).
- J. turcomanica B. Fedtsch. in Fedtschenko et al., Fl. Turkmenii 1:14 (1932).

Sabina turcomanica (B. Fedtsch.) Nevski, Trudy Bot. Inst. Akad. Nauk S.S.S.R., ser. 1, Fl. Sist. Vvss. Rast. 4: 218 (1937).

J. excelsa M.-Bieb subsp. turcomanica (B. Fedtsch.) Imkhan., Bot. Zurn. 75(3): 408 (1990).

J. polycarpos K. Koch var. pendula Mulk., Dokl. A. N. Armen. S.S.R. 45(2):86 (1967).

J. excelsa M.-Bieb. subsp. polycarpos (K. Koch) Takht. var. pendula (Mulk.) Imkhan., Bot. Zurn. 75 (3): 407 (1990).

J. excelsa M.-Bieb. var. farreana P. N. Mehra, Nucleus 19(2): 135.(1976) nom. inval. Art. 36.1.

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References

Adams, R.P., 1975a. Numerical-chemosystematic studies of infraspecific variation in *Juniperus pinchotii* Sudw. Biochem. Syst. Ecol. 3, 71–74.

Adams, R.P., 1975b. Statistical character weighting and similarity stability. Brittonia 27, 305-316.

Adams, R.P., 1991. Cedar wood oil — analysis and properties. In: Linskins, H.F., Jackson, J.F. (Eds.), Modern Methods of Plant Analysis: Oils and Waxes. Springler, Berlin, pp. 159–173.

Adams, R.P., 1995. Identification of essential oils components by gas chromatography mass spectroscopy. Allured Publ, Carol Stream, IL.

Adams, R.P., 1999. Systematics of multi-seeded eastern hemisphere *Juniperus* based on leaf essential oils and RAPD DNA fingerprinting. Biochem. Syst. Ecol. 27, 709–725.

Adams, R.P., Demeke, T., 1993. Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs). Taxon 42, 553–572.

Ciesla, W.M., Mohammed, G., Buzdar, A.H., 1998. Juniper dwarf mistletoe, Arceuthobium oxycedri (DC.)
 M. Bieb., in Balochistan Province, Pakistan. Forestry Chronicle 74, 549-553.

Demeke, T., Adams, R.P., Chibbar, R., 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPDs): a case study in Brassica. Theor. Appl. Genet. 84, 990–994.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for smallquantities of fresh leaf tissue. Phytochemical Bull. 19, 11-15.

Farjon, A., 1992. The taxonomy of the multiseed junipers (*Juniperus* sect *Sabina*) in southwest Asia and east Africa. Edinb. J. Bot. 49, 251–283.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53, 326–338.

Gower, J.C., 1971. A general coefficient of similarity and some of its properties. Biometrics 27, 857–874. Silba, J., 1986. Encyclopaedia coniferae. Phytologia Memoirs 8, 1–217.

Silba, J., 1990. A supplement to the international census of the coniferae, II. Phytologia 68, 7-78.